THE CHROMATOPHORE SYSTEM IN THE CATFISH AMEIURUS

G. H. PARKER

(From the Biological Laboratories, Harvard University)

INTRODUCTION

The color changes in the catfish, *Ameiurus nebulosus,* have attracted general attention for at least two decades, but they have been studied in detail only during the last five or six years. As receptors for such changes the eyes in this fish have been recognized by all workers. In 1917 Van Heusen showed that the innervation of the catfish skin was photosensitive and some two years ago Wykes (1938) pointed out that this sensitivity may play a part in the color changes in this fish. The pineal organ has also been suspected of influencing the chromatic activities in *Ameiurus* and the direct stimulation of chromatophores by light has been invoked by some workers to this end.

The chromatophores in *Ameiurus* appear to be all melanophores, micromelanophores in the epidermis and macromelanophores directly under the derma. Of these the macromelanophores are much the more convenient for study. The melanophores in *Ameiurus* appear to be activated through three sets of neurohumors, intermedin from the pituitary gland, acetylcholine from the dispersing autonomic nerve-fibers and adrenaline from the concentrating fibers. The first two neurohumors disperse the melanophore pigment and thereby darken the fish; the third concentrates it and thus induces blanching (Parker, 1940*a*). The object of this paper is to discuss the systematic relations of catfish melanophores, their neurohumors, and the receptors concerned with their activation.

SPECIFIC RESPONSES OF MELANOPHORES

The melanophores of *Ameiurus* respond very differently depending upon the particular conditions of the fish: presence or absence of eyes, of pituitary gland, whether the fish is in darkness or in light, and the nature of the background white or black. Many of these types of response have been recorded already by students of this subject some of whom have partly tabulated them (Abramowitz, 1936; Osborn, 1938*b*).

Table I of the present paper summarizes my own observations on the tint of the tail of *Ameiurus,* which is the same as that of the body generally, as well as on the tint of newly cut caudal bands under different conditions. These conditions, of which sixteen are recorded, are indicated on the left-hand side of Table I.

Table I

Tints of the tails and of the newly cut caudal bands of the catfish *Ameiurus* under various conditions: with or without eyes, with or without pituitary glands, in light on white or black backgrounds, and in darkness as pale fishes or dark ones. The scale of tints in relation to the average diameters in microns of the macromelanophores is approximately as follows: pale, 45; palish, 60; intermediate 70; darkish, 95; dark, 138; coal-black, 143.

Conditions of fishes		Tints	
		Tail	New band
Eyed			
With pituitary gland			
In light			
White background	1	Pale	Darkish
Black background	2	Dark	Coal-black
In darkness			
Pale fish	3	Pale	Darkish
Dark fish	4	Dark	Coal-black
Without pituitary gland			
In light			
White background	5	Palish	Darkish
Black background	6	Intermediate	Darkish
In darkness			
Pale fish	7	Palish	Darkish
Intermediate fish	8	Intermediate	Darkish
Eyeless			
With pituitary gland			
In light			
White background	9	Coal-black	Coal-black
Black background	10	Coal-black	Coal-black
In darkness			
Pale fish	11	Pale	Darkish
Dark fish	12	Dark	Coal-black
Without pituitary gland			
In light			
White background	13	Intermediate	Darkish
Black background	14	Intermediate	Darkish
In darkness			
Pale fish	15	Palish	Darkish
Intermediate fish	16	Intermediate	Darkish

The diameters of the pigmented areas of the macromelanophores in *Ameiurus* are about 40 microns in the concentrated state and 145 microns in the dispersed one and of the micromelanophores about 12 and 90 microns respectively. Wykes (1938) and Osborn (1938*b*) in their

studies of the catfish color changes have measured the diameters of considerable numbers of melanophores under different conditions and expressed their results in averages. My own procedure has been somewhat different. I have preferred to use adjectives as descriptive of the states of the color cells instead of cell measurements and I have distinguished in the scale of changes six steps from extreme dispersion of pigment to its extreme concentration (Table I). These steps have been judged in any given fish by the eye in comparison with the tints of other fishes kept as standards. This can be illustrated by the most difficult steps to distinguish that between dark and coal-black.

To carry out this separation standards were made in the following way. Two catfishes closely matched in color were kept in an illuminated, black-walled aquarium for several days till they had come to a uniform deep tint, dark. The eyes of one of these fishes were then removed whereupon the fish, still illuminated, quickly deepened in tint to what has been called coal-black. In the illuminated, black-walled aquarium these two fishes, one dark and the other coal-black, continued to maintain with great persistence these two tints. They could then be used as standards for estimating the colors of other experimental fishes. By appropriate steps additional fishes were standardized for other points in the scale and the fishes thus prepared were used in judging the color of fishes under test. In my opinion this method is a more delicate and certain one for determining tints than that of measuring macromelanophores.

Another feature in the measurement of melanophores should be mentioned. Catfishes of the lengths used in these studies, about 15 cm., are continually losing melanophores and forming new ones (Odiorne, 1937). Small macromelanophores are therefore presumably young ones and in process of increasing in size. As a means of comparison in the present study a considerable number of melanophores under a variety of conditions were measured, but in consequence of the way in which melanophores grow these measurements were made not on the total population of color cells but on the large and presumably mature cells. This selective process is reasonable, for what is of concern at this stage is not the diameters of melanophores in general, but the diameters of their pigment areas as seen in their dispersed and their concentrated states.

On several occasions during these studies full sets of melanophore measurements were carried through on all the conditions of catfishes noted in Table I. These consisted of micrometer eye-piece measurements on at least ten mature macromelanophores in each of three catfishes for all of the sixteen states given in the table. These measure-

ments afforded the basis for the rough estimates of the diameters of macromelanophores in relation to the tints of the fishes as stated in the heading for Table I. In general they are in line with the kind of measurements made by Wykes and by Osborn. Of these steps in color changes the one from dark to coal-black has long been known and recognized as of considerable importance. Yet its difference in terms of melanophore diameters, about five microns, is scarcely within measurable limits though it is easily noted by direct inspection. Hence, as already stated, inspection by eye and the use of standard fishes may be a more effective way of discovering color differences than measuring melanophores.

No detailed comments as to the contents of Table I will be made here, but the data in this table will be referred to frequently in the course of the following discussion. A few general remarks, however, may not be inappropriate. The fully pale fishes are those with eyes, pituitary glands, in bright light and on a white background (Table I, 1). This high degree of paleness disappears with the loss of the pituitary gland (5). The coal-black fishes are eyeless, but with pituitary glands and in the light (9, 10). Newly cut bands are coal-black only when induced in fishes which have not been hypophysectomized (2, 4, 9, 10, 12). No hypophysectomized fish either in the tint of its tail or in that of its band ever reaches full paleness or coal-blackness (5 to 8 and 13 to 16). These limitations have already been pointed out by Osborn (1938*a*), Veil (1938), Wykes (1938), Enami (1939), and Parker (1940*a*). The states in darkness recorded in the table involve resting states of melanophores (Parker, 1939; 1940*b*) and hence fishes showing the two possible extremes of tint at the time of their introduction into darkness are given, pale or dark for those with pituitary glands (3, 4, 11, 12) and palish or intermediate for those without these glands (7, 8, 15, 16).

The Activation of Melanophores

The activation of the melanophores in *Ameiurus* has been attributed to the direct action of light on these color cells or by means of such receptors as the pineal organ, integumentary photoreceptors, and the eyes. The direct action of light on the melanophores of the catfish was suggested as early as 1934 by Parker. It was tested experimentally by Wykes (1938) who found that a narrow beam of light focused on the skin of a catfish by means of a lens was not followed by any chromatophoral response. I have also tested this matter experimentally. The tail of a catfish of intermediate tint was split longitudinally into halves by an incision running from the edge of the tail to its root and parallel

with its rays.   A fish with a tail thus split was tied on its side to a black board and provided with a current of fresh water through its mouth and gills for respiration.   The split tail was then pinned out on the board with one half of it freely exposed and the other half covered with a light-proof, metal chamber so fitted into the board and about the tail that the portion of the tail within it was entirely inaccessible to light. The whole preparation was set up in a dark room and the exposed half of the tail was illuminated by a beam of light from an otherwise completely obscured electric lamp.   Thus on the same fish one half of the tail was brightly illuminated and the other half was in full darkness. The fish, which remained normally alive and active under these conditions, was held in this position for a little over an hour.   Its tail was then cut off, immediately fixed expanded in hot water at 60° C. and prepared for microscopic examination.   In all, three such preparations were made.   To the unaided eye the two halves of the tail in each of these were indistinguishable in tint and a microscopic examination of their melanophores showed no consistent differences.   I therefore agree with Abramowitz (1936) and with Wykes (1938) that there is thus far no evidence in favor of the direct response of the melanophores in *Ameiurus* to light and I regard this possible factor as negligible in dealing with the color changes in this fish.

Since the appearance of papers by von Frisch (1911) and by Scharrer (1928) the pineal organ has been an object of interest to students of the color changes in the lower vertebrates.   Wykes (1938) opened the cranium of *Ameiurus* from the dorsal side and destroyed the pineal organ and a part of the diencephalon with a hot needle, but found that after the fish's recovery its color responses to light persisted normally. She pointed out that the roof of the skull and the skin in *Ameiurus* were so dense that the brain seemed well protected from light.   She therefore doubted if the pineal organ in *Ameiurus* was functionally concerned with color changes.

Although the roof of the cranium of the catfishes with which I worked was very dense, as had been remarked by Wykes for her own material, this part of the catfish is by no means impervious to light, as can be seen by holding a preparation of the roof of the head between one's eye and an electric light.   To prepare catfishes for pineal tests I cut the integumentary nerves to the top of the head to eliminate the possible action of photoreceptors in the skin of that part and then loosened the skin over the head so that a light-proof, thin, metal sheet could be slipped under the skin to cover fully the pineal region.   Such fishes were then subjected to bright local illumination over the pineal organ with and without the light-proof sheet in place.   In none of these tests did I

notice any change in tint in the experimental fish as compared with a second fish bound next to the one tested and used as a control. I therefore conclude that however the pineal organ may function in the color changes of other fishes, it gives no evidence in *Ameiurus* of being concerned with chromatic responses. My opinion is thus in agreement with that of Wykes.

It will be remembered that some years ago Kropp (1929) pointed out that aqueous extracts of the eye-balls of dark-adapted tadpoles of *Rana clamitans* yielded a mixture that when injected into other tadpoles induced an expansion of their melanophores. To test this possibility in the catfish I ground to a pulp in a mortar with sand and 2 cc. of Ringer's solution ten pairs of eyes from dark catfishes, extracted the juice and injected 0.5 cc. of this juice into a pale catfish and an equal amount into a dark one. I also made a similar extract from the eyes of ten pale catfishes and injected equal volumes of this extract into a pale and into a dark fish. Careful inspection, however, showed no change whatever in the colors of the recipients either local or general and I was forced to conclude that the eye-balls of *Ameiurus* were probably not concerned in any way as centers for the production of hormones of significance in color changes.

This negative evidence from direct stimulation, the activity of the pineal organ, and of the eye-balls as secretory centers is in strong contrast with the positive results from the two other means of activation, the eyes and the integumentary photoreceptors. All workers on the color changes of catfishes have recognized the great importance of the eyes in these responses. Moreover, the necessary presence of another type of receptor, such as the integumentary photoreceptor, has been repeatedly emphasized (Abramowitz, 1936; Matsushita, 1938; Wykes, 1938). To distinguish in an experimental way the parts played by the eyes and the skin receptors in *Ameiurus* is by no means easy. One operational procedure to this end has been found possible. It consists in a complete transverse section of the brain of the catfish in the region of the cerebellum. When such a section is made the portion of the brain anterior to it carries the eyes, the pituitary gland and their connections undisturbed and no other organs of significance for color changes, for the only other components connected with this part of the brain are, in addition to the pineal organ just discussed, the olfactory nerves and the nerves to the eye-muscles. The portion of the central nervous organs posterior to the cut, the hind part of the brain and the whole of the spinal cord, has attached to it all the cutaneous nerves, from the trigeminal to the last spinal. Thus this cut separates completely and perfectly the eyes and their immediate central connections from the nerves

to the whole of the skin and their immediate centers. Such a preparation has its limitations, but is of great importance in determining the rôles played by the two chief systems of chromatic receptors at present under consideration.

The operation for the transverse severance of the brain of the catfish is carried out without great difficulty. The cut is made in a plane transverse to the longitudinal axis of the fish and a little posterior to the anterior edges of the pectoral fins. In executing this cut in a catfish a small aperture is bored through the skin and bony roof of the cranium of the fish on the dorsal median line and in the transverse plane already described. A scalpel with a very narrow, long blade is then inserted through this aperture and with one transverse cut to the right supplemented by a second one to the left the brain may be completely severed cross-wise. In the catfish the brain is not always uniform in location in reference to external landmarks. A transverse cut through the brain, however accurately made, may therefore not always fall in the desired region. If the cross-cut is a millimeter or so too far anterior, the whole optic and pituitary organization may be interfered with and the preparation thus rendered worthless. If it is too far posterior, it may disrupt the medulla oblongata and disturb the respiratory centers to such an extent that the fish will quickly die. If, however, the cut is successful, the desired separation in the brain may be satisfactorily accomplished and the fish after a few days will be ready for tests. I was fortunate in this operation in about one in every four or five fishes. This rather heavy loss was due, I believe, to the considerable variation in the position of the brain in relation to the external topography of the fish as already noted. Fishes successfully operated on commonly lived for from three to four weeks. They were quite active, but their swimming was disorganized and their orientation completely lost. These motor disturbances were doubtless due to the partial destruction of the cerebellum. Such fishes were, however, well adapted as material for chromatic tests.

A pale catfish, when operated upon as already described, will remain pale apparently indefinitely even in a black-walled, illuminated aquarium. One of my fishes lived thus for 24 days after its brain had been transected and died finally because of further operations. As might be supposed, these fishes remain indefinitely pale on illuminated, white backgrounds. Since, however, they stay pale in black, illuminated surroundings and with their eyes, pituitary glands and the connections of these parts fully intact, one is justified in concluding that the eyes of the catfish are not the receptors concerned with the discharge of intermedin from the pituitary gland.

Dark fishes with brains transected through the cerebellum remain dark irrespective of their surroundings and in their unchangeableness resemble pale fishes. The brain transection, in fact, appears to reduce both kinds of fishes to a stage of color immobility. They are in truth, as I have elsewhere shown (Parker, 1940*b*), in a state of chromatic rest. This brain operation appears to render the whole melanophore system of the catfish inoperative. That the melanophores of such fishes are not injured by the transection but are merely rendered quiescent can be shown by the injection of intermedin into a pale operated catfish whereupon it will darken, and the injection of adrenaline into a dark one which will then quickly blanch. These observations lead to the conclusion that the eyes of the catfish are not concerned with the discharge of intermedin.

Further evidence as to the control of intermedin in catfishes can be obtained from individuals whose central nervous organs are intact. When both eyes are removed from a normal, pale catfish and it is allowed to remain in the light, it soon assumes a coal-black tint irrespective of its background (Table I, 9, 10). Such a fish will apparently retain this maximum dark hue for an indefinite period (Van Heusen, 1917; Bray, 1918; Pearson, 1930; Parker, 1934; Abramowitz, 1936; Odiorne, 1937; Wykes, 1938; Osborn, 1938*b*). If a coal-black fish, rendered so by having been blinded, is transferred to darkness, it will cease to be coal-black, but will maintain a very dark tint (Table I, 12).[1] If a pale fish is completely enucleated and put at once into the dark, it will remain pale (Table I, 11). Both these fishes, however, when brought into the light soon become coal-black. I have shown elsewhere (Parker, 1940*b*) that the condition of the melanophores of such fishes in darkness is that of rest and that this stage of rest may be assumed anywhere between one extreme or the other in the whole range of color change. This stage of melanophore inactivity in darkness shows that the photoreceptors in the skin of such fishes are also in quiescence, for all such *Ameiurus* when brought into the light darken to full coal-black. In darkness, then, the photoreceptors in the skin of the catfish must be unstimulated, but when light falls upon them they are activated and thus induce the discharge of intermedin whereby the fish assumes a coal-black tint. That this darkening is due to a blood-borne agent is seen in the fact that when this test is carried out on a fish with a blanched, caudal band, the band darkens at the same time that the fish does.

[1] This statement is not wholly in agreement with the declarations of other workers. I have discussed these differences elsewhere (Parker, 1940*a*) and I shall refer the reader to this discussion for my reasons for the conclusion that blinded, coal-black catfishes in darkness are of very dark tints though not coal-black.

These observations lead to the conclusion that when light falls upon the integumentary photoreceptors of *Ameiurus* nervous impulses are set up for the discharge of intermedin and the consequent darkening of the fish (Abramowitz, 1936). They also show that these photoreceptors are fully quiescent in darkness. I therefore conclude in general that the discharge of intermedin is not the result of impulses from the eyes, but of impulses from the integumentary photoreceptors and that these receptors are stimulated to this effect when light falls upon them but not when they are in darkness.

From this standpoint the one chromatic activity of the photoreceptors in the catfish skin is to induce the discharge of intermedin. That this general control is a reflex operation there is every reason to assume. That it is not a nervous, spinal-cord reflex is clear, for no changes in color can be induced in a spinal catfish by the illumination of the skin on its flanks. In this my observations entirely agree with those of Wykes (1938). However, I do not agree with this worker in assuming that the reflex under consideration is a brain reflex in the way implied by her. Wykes assumes a type of reflex based upon an interpretation of the expanded state of melanophores in denervated pelvic fins whereby the color cells therein located are supposed to be in a condition of dispersed pigment in consequence of their liberation by the denervating cut from some central nervous activity. But this interpretation has already been shown to be untenable (Parker, 1934, 1940a). It is true that the reflex here discussed is, as Wykes remarks, a reflex that involves the brain, but the activating impulses are not reflected from that organ over nervous paths to the melanophores as she believes. As already shown, they impinge in the brain on the pituitary gland there to induce the discharge of intermedin. In this type of reflex only the afferent arm is nervous (from the integumentary photoreceptor to the pituitary gland), the efferent arm being humoral (from the pituitary gland by way of the blood and the lymph to the melanophore). I have recently called attention to this somewhat unusual type of reflex, part nervous and part humoral, and I have designated it in consequence of the different conditions of its two arms as a neurohumoral reflex (Parker, 1940c; 1940d). In conclusion it may be said that the discharge of intermedin by the pituitary gland in *Ameiurus* according to this general view is not excited through the eyes, but through the photoreceptors in the skin and by these photoreceptors only when they are illuminated, not when they are in darkness. Under such circumstances they are inactive.

If the skin photoreceptors in *Ameiurus* are limited in their chromatic function to exciting a discharge of intermedin from the pituitary

gland as a means of darkening the fish, what part do the eyes play in these color changes? It has already been shown that the eyes are not concerned with the discharge of intermedin, for a pale catfish with a transected brain but with eyes and pituitary gland intact will remain pale on a black, illuminated background. Do the eyes control the autonomic nerve-fibers by which the melanophores are made to disperse or to concentrate their pigment?

This question has been in large part answered by the work of previous investigators. The presence of dispersing fibers in the catfish, notwithstanding the doubtful expressions voiced by Osborn (1938b) and by Wykes (1938), appears to be well established on the basis of the revival of caudal bands (Parker, 1934; 1940a) and of the regeneration of caudal pigmentary nerves (Abramowitz, 1935). Dispersing fibers have been taken for granted in two of the most recent papers on this subject (Chin, 1939; Chang, Hsieh and Lu, 1939). Are these fibers activated from the photoreceptors in the skin, from the eyes or from both sets of receptors? This question cannot be approached from the standpoint of the fish with transected brain, for the cut in such an animal severs not only the nervous tracts from the skin to the pituitary gland, but also those from the eyes to the melanophores. It is best dealt with in hypophysectomized catfishes. Hypophysectomized catfishes lose the intermedin from their blood only slowly. According to Veil (1937) they blanch in about three days. Osborn (1938b) identified intermedin in the blood of catfishes by means of tests on pale fishes 70 hours after hypophysectomy. By testing the blood of hypophysectomized fishes on pale individuals or on catfishes of intermediate tint, I have found evidences of intermedin at three days after hypophysectomy but never after five days. When catfishes completely free from intermedin were needed for experimental purposes they were held as a rule for a full week after hypophysectomy before special tests were made upon them. As already stated, a hypophysectomized catfish thus seasoned is limited in its color responses. It is incapable of becoming fully dark (Parker, 1934; Osborn, 1938a, 1938b; Wykes, 1938; Enami, 1939) or fully pale (Veil, 1938; Enami, 1939; Parker, 1940a). In normal *Ameiurus* the fully dispersed macromelanophores, as already noted, have a diameter of about 145 microns and the fully concentrated cells one of about 40 microns. After hypophysectomy the diameter of these cells at maximum dispersion is about 70 microns according to Osborn (1938b) or about 100 microns according to my measurements (Parker, 1940a) and their minimum diameters some 50 microns. Thus by hypophysectomy the catfish has lost about half its range in color change. The half range that remains to the fish after the loss of its

pituitary gland must be attributed to its other chromatic activators which are in the main the nerves for the dispersion and the concentration of its pigment.   The dispersing nerves, supposed by some workers (Parker, 1934; Wykes, 1938) to be the chief agents in darkening the fish, have been shown to be of secondary importance as compared with intermedin (Veil, 1937; Osborn, 1938a, 1938b; Parker, 1940a).   The concentrating nerves have long been recognized as the main blanching agents. These general features of the catfish melanophore system must be kept in mind in the following discussion which will be concerned chiefly with the steps necessary for the activation of the two sets of nerves for the color responses.

If a catfish previously hypophysectomized is completely enucleated the system controlling its melanophore responses will be found to be reduced to the photoreceptors of the skin and their nerves, and the two sets of dispersing and concentrating melanophore nerves.   These parts together constitute all that is necessary for a complete melanophore reflex.   Are these parts so organized that such a reflex is possible?

Catfishes prepared in this way and allowed to recover fully from the operations live well for some weeks.   They are very satisfactory material for color tests.   Their general conditions are recorded in Table I, 13 to 16.   When a hypophysectomized catfish is enucleated even though it is in the beginning as moderately pale as such a fish can be, it is likely to darken considerably as a result of the operation.   This is probably due to handling (Parker, 1940a).   After such treatment these fishes are as a rule about intermediate in tint (Osborn, 1938b; Wykes, 1938) though some of them remain paler.   In the light they retain their particular tints apparently indefinitely and this retention is irrespective of the background on which they rest (Table I, 13, 14).   If hypophysectomized catfishes as near pale and as near intermediate in tint as they can be made to assume by background adjustment are quickly and carefully enucleated and at once put in darkness, they retain their original tints without change so far as can be judged (Table I, 15, 16).   Moreover, when they are brought into the light they do not seem to alter in color.   All these observations point to hypophysectomized, eyeless catfishes as chromatically inert and open to almost no reflex color change whatever.   Their melanophores are in what I have regarded as stages of rest (Parker, 1940b).   That these color cells have not suffered any serious incapacity by the operations to which the catfish may have been subjected can be shown in their quick and characteristic responses of pigment concentration to injections of adrenaline and of dispersion to pituitary extract (Osborn, 1938a).   What is lacking apparently in these inert fishes is the reflex connections between the afferent photoreceptor

fibers from the skin and the efferent dispersing and concentrating fibers to the melanophores. In the catfish the nerve connections left after the removal of the pituitary gland and the eyes are not organized for color reflexes and I therefore conclude that the autonomic dispersing and concentrating nerve-fibers of the melanophore system in *Ameiurus* are not open to activation by the photoreceptor fibers from the skin of the fish. These two sets of melanophore fibers, as has long been known, are activated through the eyes and if the results just detailed are correct these receptors are the only ones concerned with the activation of these two kinds of nerve-fibers.

Discussion

The melanophore system in *Ameiurus,* as represented by its macro-melanophores, appears to be organized upon the following general plan. The chief receptors concerned with the activation of the melanophores are the eyes and the photoreceptors of the skin. The photoreceptors are stimulated not in darkness but only when light falls upon the skin and are not influenced by an experimental environment, black or white. They excite the discharge of intermedin from the pituitary gland and the consequent darkening of the fish. They are not concerned with the activation of the chromatic nerve-fibers, dispersing or concentrating. The eyes are receptors through which a background, white or black, makes itself felt in the system of color cells. Through the eyes are excited the concentrating autonomic nerve-fibers and the dispersing ones by which the pigment in the melanophores is gathered in the center of the color cell or scattered throughout its processes, blanching or darkening the fish. The eyes, therefore, are the receptors concerned with the discharge of adrenaline and of acetylcholine from the chromatic nerve terminals. The eyes are not so related to the discharge of intermedin, and yet it would probably be false to deny to them any influence over the pituitary gland. When a catfish becomes pale through the entrance into its eyes of light reflected from a white surface, its skin is also under reasonably bright illumination. The effect of this skin illumination would be to induce the discharge of intermedin whereby the fish would be darkened. There is, however, not the least evidence to show that in a brightly il-luminated, white environment any darkening agent is present in a catfish. On the contrary, the fish under these circumstances is fully and com-pletely pale as though under the undivided control of adrenaline. It is conceivable, of course, that there is under these conditions a neuro-humoral competition between intermedin and adrenaline like that which has already been pointed out in the dogfish *Mustelus* (Parker, 1937),

but evidence to this effect, unlike that seen in the dogfish, is entirely lacking. It is much more probable that in illuminated white surroundings the stimulation of the eye excites only the concentrating nerve fibers and at the same time overcomes the discharge of intermedin due to the illumination of the skin photoreceptors by some action on the pituitary gland. From this standpoint the eye in a luminous white environment not only excites the discharge of adrenaline but probably inhibits that of intermedin. In this respect the eye very likely plays a negative but important part in the control of intermedin. This idea of chromatic inhibition has already been suggested by Zoond and Eyre (1934) and by Sand (1935).

This, in brief, is a rough outline of the melanophore system in *Ameiurus*. That it is correct in its chief features seems probable from the evidence presented. That it is in any sense complete is far from likely. Why should hypophysectomy limit color responses and how is this limitation accomplished at both the dark and the pale ends of the color range? Is excitement pallor (Abramowitz, 1936) a reflex and if so what are the receptors and the nerve paths? The same questions may be asked concerning darkening due to handling. What is the nature of the so-called inherent reactions of melanophores, a subject recently emphasized by several active workers (Abramowitz, 1936; Osborn, 1938b) ? These and a score of other questions show how restricted our knowledge is not only of the melanophore system in *Ameiurus* but of the chromatic system in general. They invite further research.

Summary

1. The melanophore system in the catfish *Ameiurus* composed of epidermal micromelanophores and of subdermal macromelanophores is activated not through direct stimulation by light, by humors from the eye ball, nor by the activity of the pineal organ, but by nerve impulses received from the eyes and from the photoreceptors in the skin.

2. The skin photoreceptors are stimulated by light but not by darkness and are exclusively concerned with the discharge of intermedin from the pituitary gland whereby the catfish darkens (neuro-humoral reflex).

3. The eyes are differently stimulated by light from a black environment and by light from a white one. In the former they give rise to impulses that pass over the dispersing autonomic nerve-fibers, induce a discharge of acetylcholine, the dispersion of pigment, and the darkening of the fish. In the latter they originate impulses that pass over the concentrating autonomic fibers, induce a discharge of adrenaline, the con-

centration of pigment, and the blanching of the fish.   Impulses induced through the eye by an illuminated, white environment probably also inhibit the discharge of intermedin from the pituitary gland.

4. A technique is described for the transection of the catfish brain at the level of the cerebellum whereby the eyes and their appended centers may be separated from the whole integumentary photoreceptor system and its centers.

REFERENCES

ABRAMOWITZ, A. A., 1935.   Regeneration of chromatophore nerves.   *Proc. Nat. Acad. Sci. Washington*, 21 : 137–141.

ABRAMOWITZ, A. A., 1936.   Physiology of the melanophore system in the catfish, Ameiurus.   *Biol. Bull.*, 71 : 259–281.

BRAY, A. W. L., 1918.   The reactions of the melanophores of Amiurus to light and to adrenalin.   *Proc. Nat. Acad. Sci. Washington*, 3 : 58–60.

CHANG, H. C., W. HSIEH, AND Y. M. LU, 1939.   Light-pituitary reflex and the adrenergic-cholinergic sympathetic nerve in a teleost.   *Proc. Soc. Exp. Biol. Med.*, 40 : 455–456.

CHIN, Y. C., 1939.   Does acetylcholine play a part in the mechanism of melanophore expansion?   *Proc. Soc. Exp. Biol. Med.*, 40 : 454–455.

ENAMI, S., 1939.   Rôle de la sécrétion hypophysaire sur le changement de coloration chez un poisson-chat, Parasilurus asotus (L.).   *Compt. Rend. Soc. Biol. Paris*, 130 : 1498–1501.

VON FRISCH, K., 1911.   Ueber das Parietalorgan der Fische als funktionierendes Organ.   *Sitzb. Gesell. Morph. Physiol. München*, 27 : 16–18.

KROPP, B., 1929.   The melanophore activator of the eye.   *Proc. Nat. Acad. Sci. Washington*, 15 : 693–694.

MATSUSHITA, K., 1938.   Studies on the color changes of the catfish, Parasilurus asotus (L.).   *Sci. Rep. Imp. Univ. Sendai*, 4 Ser. Biol., 13 : 171–200.

ODIORNE, J. M., 1937.   Morphological color changes in fishes.   *Jour. Exper. Zool.*, 76 : 441–465.

OSBORN, C. M., 1938a.   The rôle of the melanophore-dispersing hormone of the pituitary in the color changes of the catfish.   *Proc. Nat. Acad. Sci. Washington*, 24 : 121–125.

OSBORN, C. M., 1938b.   The role of the melanophore-dispersing principle of the pituitary in the color change of the catfish.   *Jour. Exper. Zool.*, 79 : 309–330.

PARKER, G. H., 1934.   Color changes of the catfish Ameiurus in relation to neurohumors.   *Jour. Exper. Zool.*, 69 : 199–233.

PARKER, G. H., 1937.   Antagonism in neurohumors as seen in the pectoral bands of Mustelus.   *Proc. Nat. Acad. Sci. Washington*, 23 : 596–600.

PARKER, G. H., 1939.   The active and the resting states of melanophores tested experimentally.   *Anat. Rec.*, 75 : Suppl. 61.

PARKER, G. H., 1940a.   On the neurohumors of the color changes in catfishes and on fats and oils as protective agents for such substances.   *Proc. Am. Philos. Soc.* (in press).

PARKER, G. H., 1940b.   The active and the resting states of catfish melanophores tested experimentally.   *Jour. Cell. Comp. Physiol.*, 15 : 137–146.

PARKER, G. H., 1940c.   Types of animal reflexes.   *Science*, 91 : 216.

PARKER, G. H., 1940d.   Novel types of nerve reflexes.   *Proc. Nat. Acad. Sci. Washington*, 26 : 246–249.

PEARSON, J. F. W., 1930.   Changes in pigmentation exhibited by the freshwater catfish, Ameiurus melas, in response to differences in illumination.   *Ecology*, 11 : 703–712.

SAND, A., 1935.   The comparative physiology of colour response in reptiles and fishes.   *Biol. Rev.,* **10** : 361–382.

SCHARRER, E., 1928.   Die Lichtempfindlichkeit blinder Elritzen.   (Untersuchungen über das Zwischenhirn der Fische I.)   *Zeitschr. vergl. Physiol.,* **7** : 1–38.

VAN HEUSEN, A. P., 1917.   The skin of the catfish (Amiurus nebulosus) as a receptive organ for light.   *Am. Jour. Physiol.,* **44** : 212–214.

VEIL, C., 1937.   Hypophysectomie et changement de couleur chez le poisson chat.   *Compt. Rend. Soc. Biol. Paris,* **124** : 111–113.

VEIL, C., 1938.   Evaluation de la quantité d'intermédine contenue dans l'organisme du poisson-chat.   *Compt. Rend. Soc. Biol. Paris,* **127** : 42–43.

WYKES, U., 1938.   The control of photo-pigmentary responses in eyeless catfish.   *Jour. Exper. Biol.,* **15** : 363–370.

ZOOND, A., AND J. EYRE, 1934.   Studies in reptilian colour response. I. The bionomics and physiology of the pigmentary activity of the chameleon.   *Phil. Trans. Roy. Soc. London, Ser. B,* **223** : 27–55.